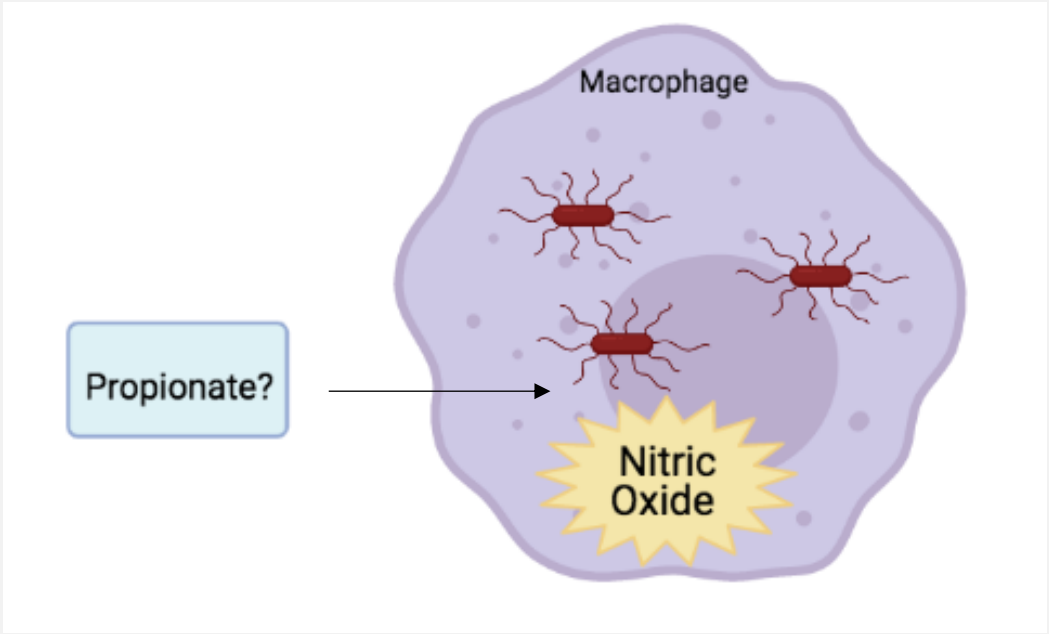
The effects of propionate on the activation of macrophages against the intracellular pathogen *Listeria monocytogenes*

Leah Allen

Research Advisor: Dr. Yvonne Sun



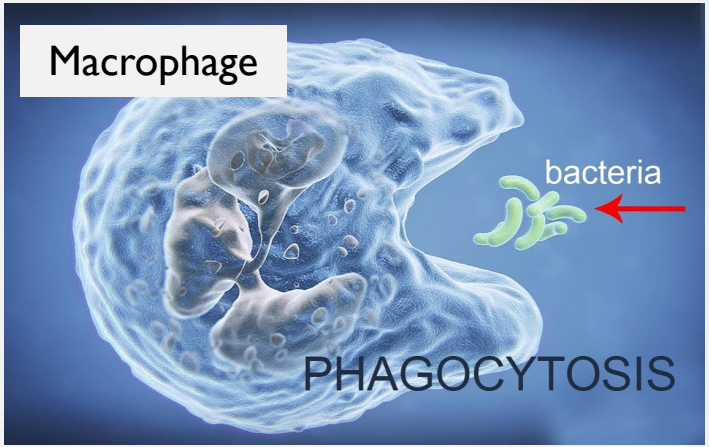
Main Research Question:

Can propionate change the antimicrobial functions of macrophages against intracellular pathogens?

MACROPHAGE

- Macrophages are phagocytic immune cells that engulf bacteria

- Intracellular pathogens such as *Listeria monocytogenes* can grow in cytoplasm of macrophages



Activated Macrophages

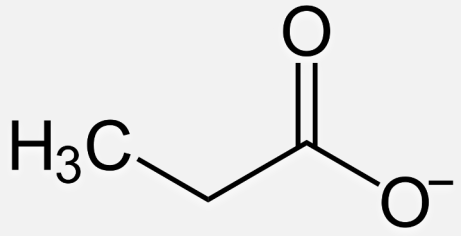
- Kill pathogens by preventing vacuole escape

- Produce reactive oxygen species and reactive nitrogen species to prevent pathogen escape into cytoplasm

NITRIC OXIDE

- Produced within macrophages by an inducible nitric oxide synthase (iNOS)
- NO can directly damage bacterial enzymes and decrease their fitness and growth
- Inflammatory molecule
- Nitrite oxide production is correlated with increased antimicrobial activity of macrophages

PROPIONATE



- Short chain fatty acid
  - Main SCFAs in gut: Acetate, Butyrate and Propionate
- Metabolite produced in gut from digestion of fibers
- Anti-inflammatory effect
- Has been shown to regulate microbial activity
  - Leads to a decrease NO production

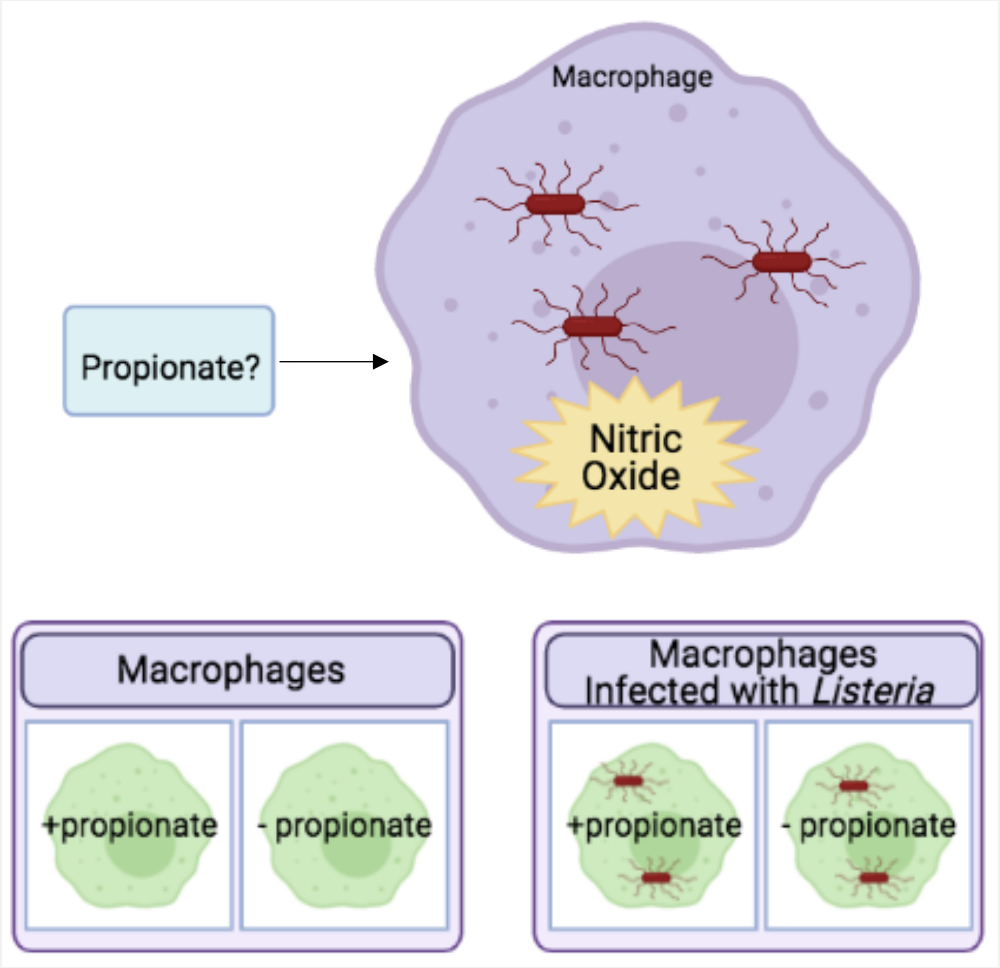
LISTERIA MONOCYTOGENES

Listeria monocytogenes

Foodborne pathogen
Listeriosis

Approx. 1,600 cases yearly (US)

1 in 5 cases result in death

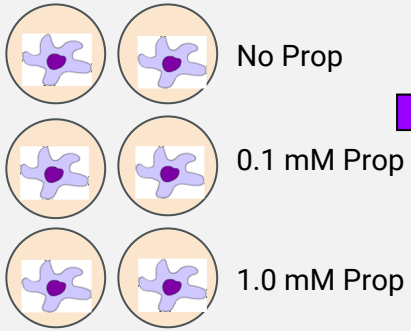


Main Research Question:

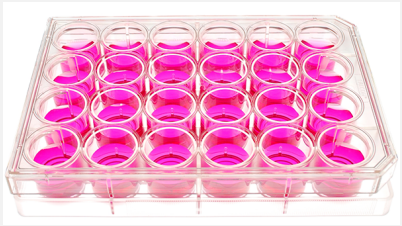
Can propionate change the antimicrobial functions of macrophages against intracellular pathogens?

RESEARCH METHOD #1: NITRITE ASSAY

Naive Activated

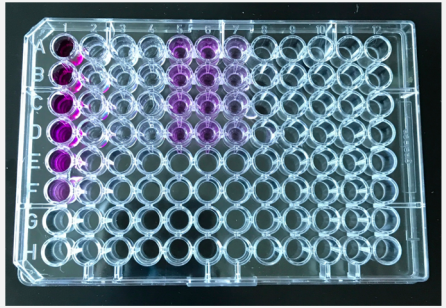


Macrophages seeded overnight in 24 well plate with propionate treatments and (LPS and IFN-γ) to activate cells



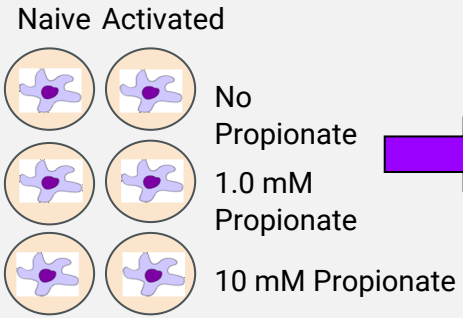
Next day, supernatant from cells were transferred to a 96 well plate

Griess Reagent

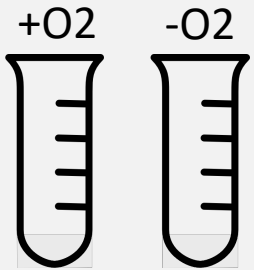


Absorbance measured at 560 nm

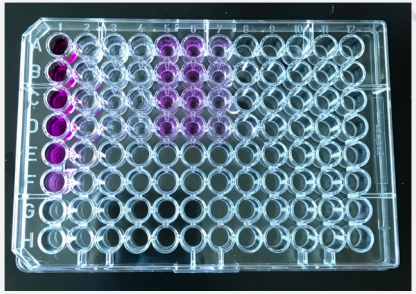
RESEARCH METHOD #2: GENTAMICIN PROTECTION ASSAY (INFECTION)



Macrophages treated with propionate for 3 hours

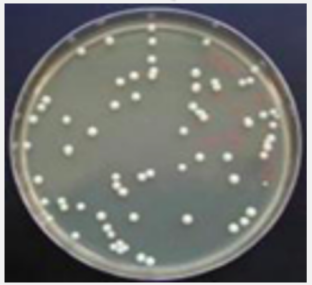


30-minute infection with aerobically and anaerobically grown *Listeria*



Nitrite assays performed at 2 and 24 hours post infection

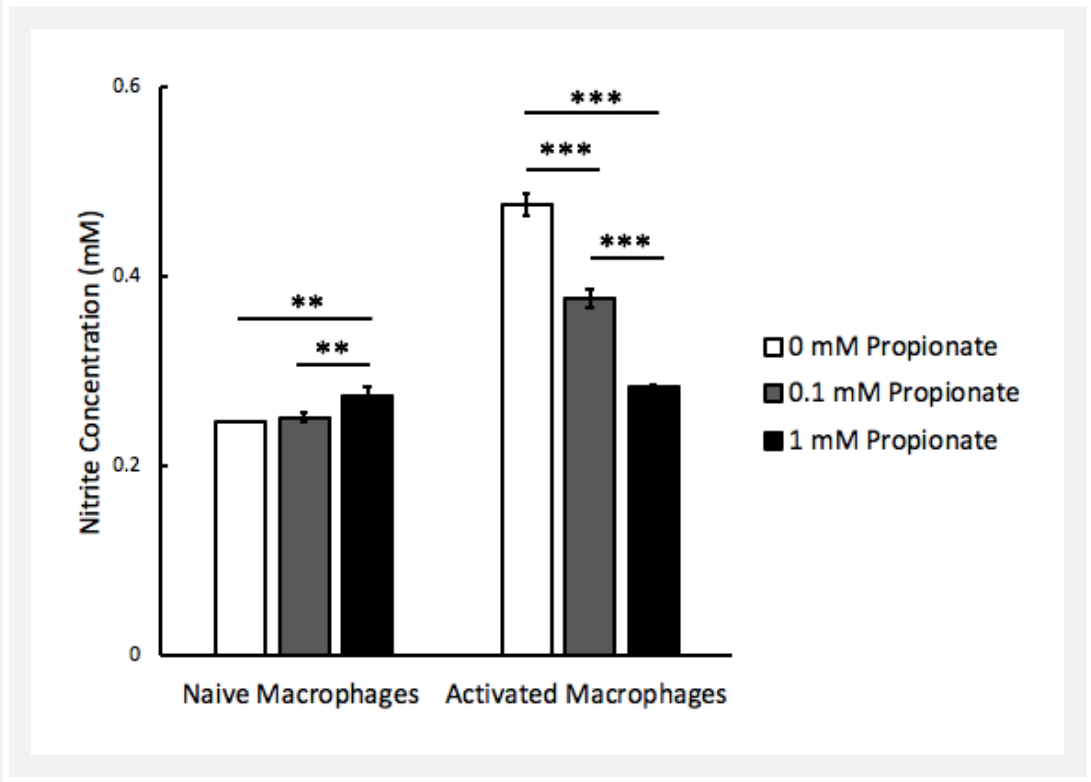
24 hpi



cells lysed and plated at 24 hours post infection

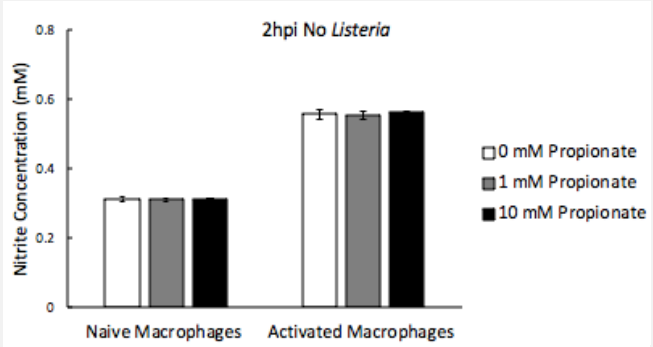
RESULTS- NITRITE ASSAY

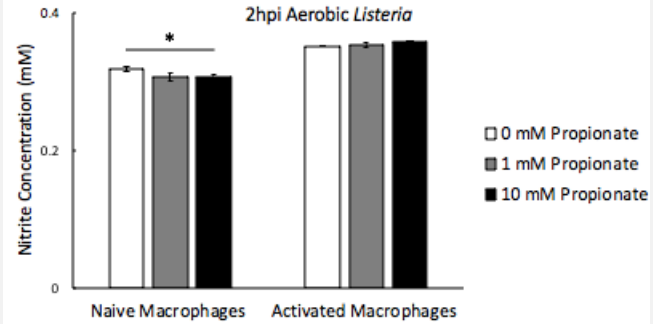
- Nitric oxide production (antimicrobial activity) was enhanced with propionate in naive macrophages
- Nitric oxide production was suppressed with propionate in activated macrophages

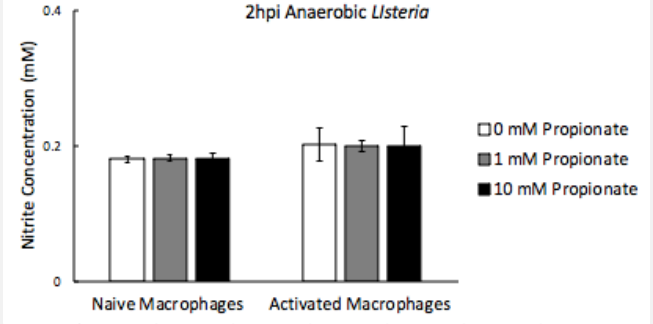


NITRITE ASSAY AT 2 HOURS POST INFECTION

- 2 hours post infection is not enough time for nitrite oxide to accumulate

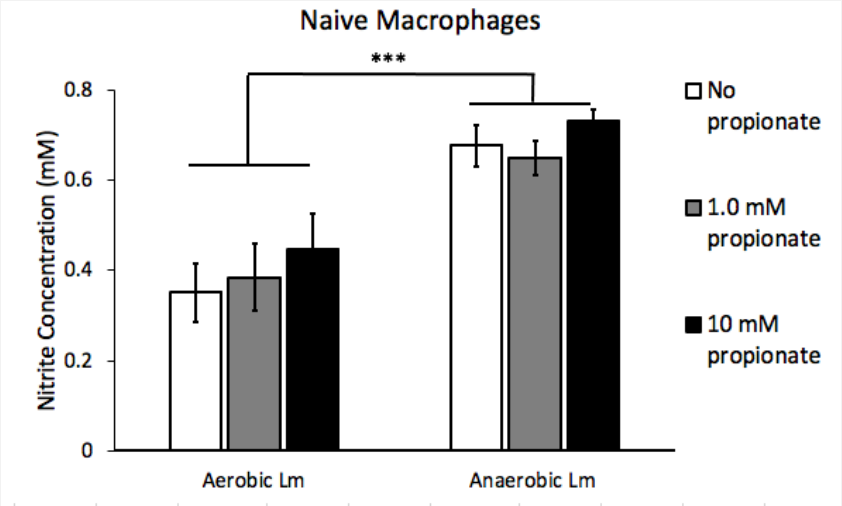


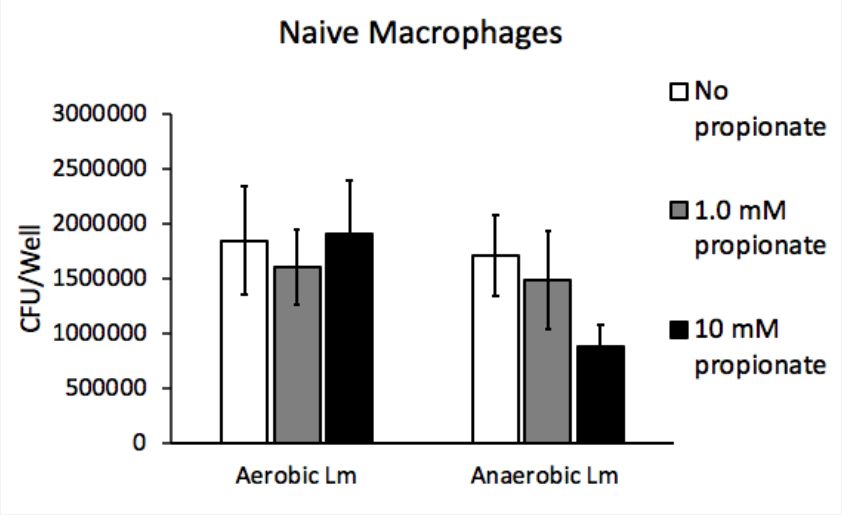




RESULTS AT 24 HOURS POST INFECTION- NAIVE MACROPHAGES

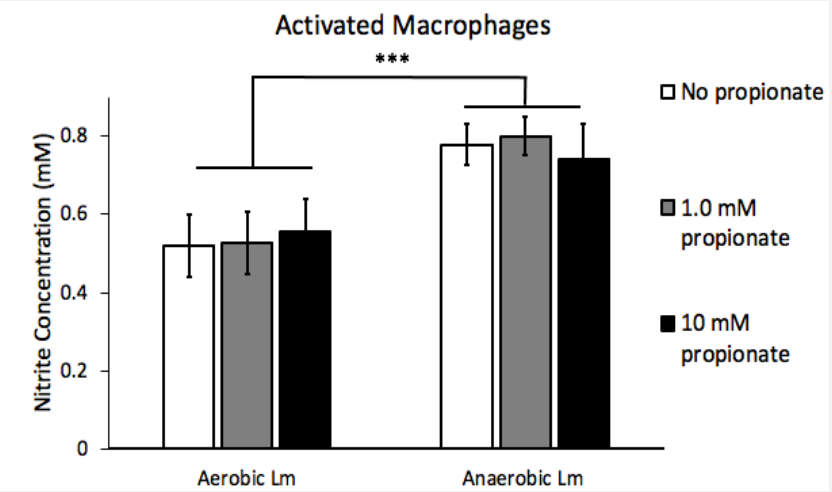
- There was a significant increase in antimicrobial activity of macrophages that were infected with anaerobically grown *Listeria*
- Propionate treatment had no significant effect on the CFU/well of macrophages infected with both aerobically and anaerobically grown *Listeria*

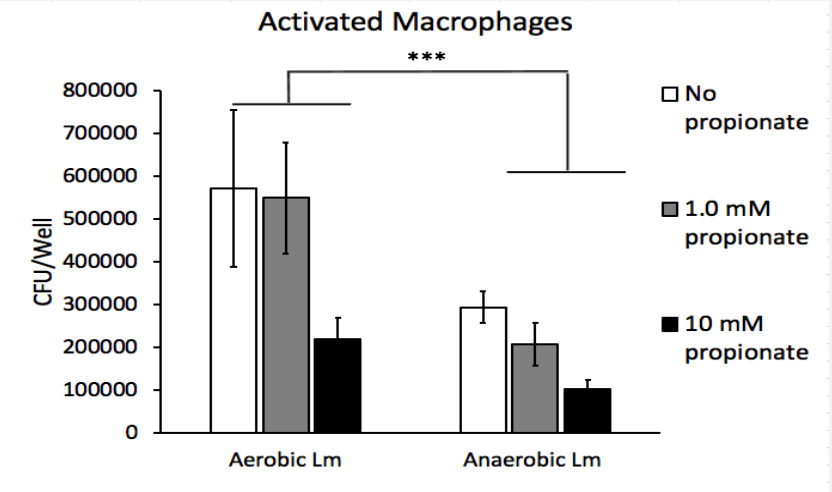




RESULTS AT 24 HOURS POST INFECTION- ACTIVATED MACROPHAGES

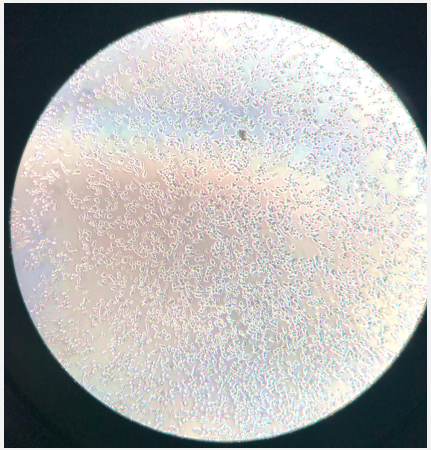
- Propionate treatment significantly increased the antimicrobial activity of macrophages infected with anaerobically grown *Listeria*
- Less *Listeria (*CFU/well) is present in activated macrophages that were treated with propionate



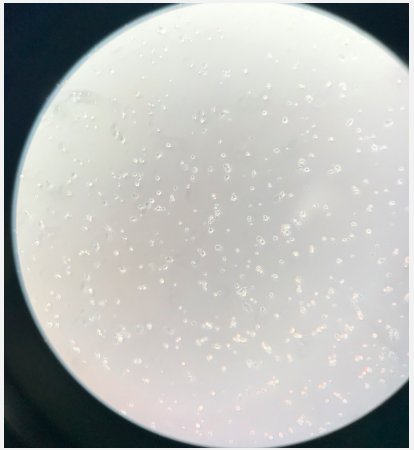


PICTURES OF MACROPHAGES 24 HOURS POST INFECTION

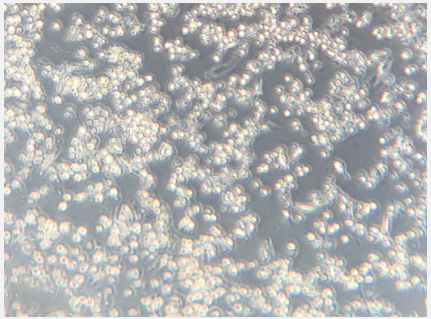
- At 24 hours there seems to be a decrease in density of activated macrophages
- There is a change in shape with propionate treatment



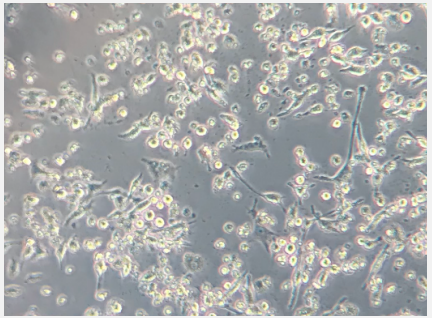
Naïve macrophage infected with aerobic *Listeria*



Activated macrophage infected with aerobic *Listeria*



Naïve macrophage no propionate



Naïve macrophage +10 mM propionate

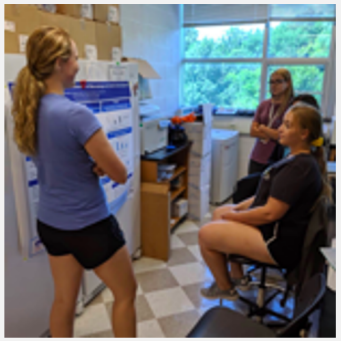
DISCUSSION

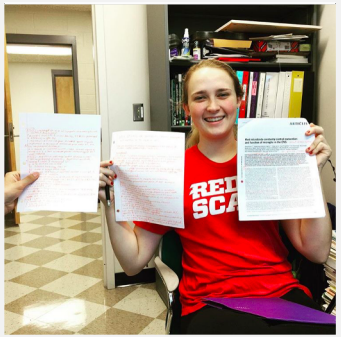
- Propionate treatment of naive macrophages resulted in increased nitric oxide production (more antimicrobial activity)
- Propionate treatment of activated macrophages resulted in decreased nitric oxide production (less antimicrobial activity)
- 3hr treatment with propionate is not enough time to observe differences in the nitrite production of macrophages
- There is significantly less *Listeria* in activated macrophages then in naïve macrophages
    - Are differences due to decreased density of activated macrophages or due to their change in shape?

CURRENT AND FUTURE RESEARCH PLANS

- Continue nitrite assay/infection experiments with propionate, naive and activated macrophages
- Further analyze pictures to determine changes of macrophage density and shape with activation and propionate treatment
- Measure iNOS expression of macrophages with qRT-PCR
- Publish research paper with other lab members

@udmicrobiology





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